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Review Article

Environmental Variables Associated with Distribution of Canine Visceral Leishmaniasis in Dogs in Ardabil Province, Northwestern Iran: A Systematic Review

Eslam MORADI-ASL [1], Mehdi MOHEBALI [2], Yavar RASSI [3], Hassan VATANDOOST [3], Abedin SAGHAFIPOUR [4]

1. *Department of Public Health, School of Public Health, Ardabil University of Medical Sciences, Ardabil, Iran*
2. *Department of Medical Parasitology & Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
3. *Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*
4. *Department of Public Health, Faculty of Health, Qom University of Medical Sciences, Qom, Iran*

***Corresponding Author:** Email: moradiasl83@yahoo.com

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Abstract

Background: Visceral leishmaniasis (VL) is a zoonotic disease that currently occurs in some parts of Europe, Asia, Africa, and America. This study aimed to determine the distribution of the canine visceral leishmaniasis in dogs (*Canis lupus familiaris*) in northwestern Iran.

Methods: The data were collected from 1994 to 2018 in Ardabil Province from electronic databases. An extensive literature search was conducted in different international and national databases, including Cochrane, MEDLINE/PubMed, Scopus, Web of Science, and Iran Medex to find articles with the words "visceral leishmaniasis in Ardabil" in their titles, and "canine visceral leishmaniasis in Ardabil" or "accidental reservoir hosts of visceral leishmaniasis in Ardabil" in their subtitles, irrespective of the type and duration of study. The GIS software and MaxEnt model were used to determine the ecologically suitable niches for the disease.

Results: In total, 9088 dogs were examined, and the overall prevalence rate of CVL in dogs was estimated to be 14.56%. The most ecologically suitable areas of CVL occurrence were identified in four hotspots in Meshkinshahr, Germi, and two spots in Parsabad counties. The results of jackknife test showed that the environmental and climate variables with the highest gain, when used in isolation, were Isothermality, Bio3, Bio13, and Bio 4.

Conclusion: A widely epidemic CVL has emerged among dogs, making a lot of risks on inhabitants of this area and increasing the probability of an outbreak of VL in humans.

Keywords: Canine visceral leishmaniasis; Dogs; Iran

Introduction

Human visceral leishmaniasis (HVL) and canine visceral leishmaniasis (CVL) are the zoonotic diseases The agents of the disease group are *Leishmania donovani* and *L. infantum* in the old world and *L. chagasi* in endemic foci in the new world (1). Its main causative agent is *L. infantum*, and phlebotominae sand flies are considered as vectors and canine are main reservoir hosts of the disease (2, 3). In addition, the domestic and stray dogs are the main reservoir hosts for VL in Iran and the world (4). The incubation period of the disease in animals' reservoir is highly variable from two months to seven years and it is depends on the virulence of the parasite and the host's genetic susceptibility (5, 6).

The accuracy of molecular diagnostic methods for CVL is very high (7). In non-endemic areas, the prevalence of CVL estimated by molecular methods is more than the estimation by serological methods (8). The most important diagnostic methods used for the diagnosis of VL include the following: indirect immunofluorescence assay (IFA), direct agglutination test (DAT), enzyme-linked immunosorbent assay (ELISA), dipstick rK39, and latex agglutination (9). Of these methods, the most frequent method used in developing countries is DAT (10). The DAT method is a simple test that has high sensitivity and specificity, low cost, and suitable performance in the field (11). The CVL is usually a chronic disease and does not has clear symptoms. The clinical symptoms in dogs may vary and often begins with a slight, yet progressive and quiet, illness. About 90% of infected dogs have skin lesions, alopecia, desquamation, ulcerative dermatitis, nodular, and pustular dermatitis forms (12).

The HVL and CVL are the zoonotic diseases that currently occur in some parts of Europe, Asia, Africa, and America (13). Currently, CVL cases are reported from over 50 countries mostly located in Eastern Mediterranean and Northern America (14). In Iran, this disease is a Mediterranean type, and it is endemic in some northwestern and southern areas, with 100-300 new cases being reported every year (15). The prevalence of CVL in different parts of the world ranges from 2.58% to 26.6% (16-18). In Iran, these values were 14.2%-17.4% (19) and the infection rate was also reported to be high in dogs without symptoms (20). There is a positive correlation between VL in humans and infected dogs, so it is an important risk factor for HVL in endemic areas of domestic and owned dogs (21). The first case of CVL in Iran was reported from Tehran in 1913 (22), and it was followed by numerous studies conducted in endemic areas by Iranian researchers. In addition, studies in Ardabil, East Azerbaijan, Qom, Chaharmahal and Bakhtiari, Khuzestan, and Bushehr provinces (23). Most of these studies were carried out on the asymptomatic dogs, domestic dogs, and asymptomatic/symptomatic dogs in Ardabil, East Azerbaijan (21), and Meshkinshahr (24-27).

Geographical and climatic conditions of Ardabil Province are suitable for developing the population of sandflies as visceral leishmaniasis vectors (3). By investigating the correlation between environmental and climatic factors with the incidence of disease in dogs as the main animal reservoirs, prevention and surveillance programs can be established in areas where susceptible to disease incidence. It also identified the areas with the highest reported parasitic contamination in animal reservoirs and determined the environmental and climatic conditions of those areas. To this end, the MaxEnt model is very useful and has been used in many infectious diseases to predict the correlation between disease incidence and environmental and climatic factors (3, 28). The Maxent model roughly calculates the spatial distribution probability of a species and its relationship to environmental variable constraints. In addition, it tries to predict the domain suitability for a species (29).

Accordingly, the following study was designed and conducted to investigate the infection of dogs as the main reservoir of VL in important endemic area of Iran in the past years and predict the current situation of this disease in humans.

Materials and Methods

Study area

Ardabil Province is located in the northwestern region of Iran (37.45-39.42 latitude and 47.30-48.55 longitude). The climate is very variable in this province. Ardabil is about 2/3 textured mountainous with a large variation in height and the rest is composed of flat, low-height areas. The main proportion of residents in this province are farmers working in farms of animal husbandries. There are also some tribes dwelling in the northern and central regions (28).

Data collection

This study systematically searched all the earlier published studies, reports, and documentations related to VL and CVL that used parasitological (microscopy and culture), serological (DAT), and molecular methods for identification and diagnosis. International databases such as Cochrane, MEDLINE/PubMed, Google Scholar, Science Direct, Scopus, Web of Science, Veterinary Information Network, VetMed Resource, Zoological Records, Biological Abstracts, and CAB Abstracts were searched with no time boundary. Moreover, Iranian databases (for Persian articles) including Iran Medex, Scientific Information Database (SID), and Magiran were also explored. The results were qualitatively summarized to find out any precise information related to the purpose of the study. All the studies which checked the status of CVL in dogs by the DAT test and were conducted in Ardabil Province were under the focus of this study. Dogs with positive serologic DAT test were included in the study. The collected data on the scale of city and villages for a spot in the Arc mapping layer were saved. The data were extracted from articles based on the following: author(s), time of study, publication year, demographic information such as age, sex, breed, geographical region of study, number of examined dogs, number of seropositive cases, and prevalence rate. The search was performed using such terms as follows: dog, leishmaniasis, leishmaniasis, Iran, canine, canine leishmaniasis, dog visceral leishmaniasis, *Leishmania infantum*, anti-*Leishmania* antibody, and epidemiology. In this study, all the data belonging to symptomatic and asymptomatic dogs with an antibody titer ≥1:320 were recorded. This value is a cut-off point for VL infection.

Data analysis

ArcGIS software ver. 9.3 (http://www.esri.com/arcgis) was used.

Mapping and Modeling

The distribution of CVL infection was mapped according to the obtained data in different counties of the study area. The Max Ent 3.3.3 (29) was used to predict the environmentally suitable areas for VL-infected dogs in Ardabil province. To do so, the coordinates of all studied areas were obtained from the national database of villages, recorded in Excel, and then converted to CSV format to be used in the model. The raster format of 19 environmental variables, including weather factors, were downloaded from the WorldClim (www.worldclim.org) website with spatial resolution of 30 sec and were clipped using the Ardabil Province mask in ArcMap 10.3. The digital elevation model (DEM) of the study area with the same spatial resolution was obtained from the National Cartography Organization, and 3 variables of elevation, slope, and aspect were derived from DEM using spatial analyst of ArcMap. Vegetation Index (NDVI) was obtained from the MODIS image. All these layers (including 22 variables) were then converted to ASCII format in ArcMap to be used later in the MaxEnt model. Eighty percent of the occurrence points of infected dogs were used in random for model training and 20% for testing the output. The Jackknife test was used to find the contribution of different variables in the model.

Results

In general, 23 articles on CVL were found in Ardabil province in 23 years (1994 to 2018). Most of the published researches (13% of studies) in 2013 were put into investigation. The most collected articles were related to the prevalence of symptomatic and asymptomatic CVL in dogs.

Overall, 9088 cases of domestic and stray dogs (72.6% of males and 27.4% females) were studied. Out of which 1671 dogs (14.56%) had DAT titer ≥ 1: 320. The infection rates in these previous studies ranged from 7.4% to 45%. Based on the results obtained by DAT method, the prevalence of CVL were 21% in Meshkinshahr, 8.25% in Germi, 6.25% in Parsabad, and 5.1% in Khalkhal and Ardabil County (Fig. 1). The highest infection rate (45%) was recorded in 2013 (Table 1).

Table 1: Baseline characteristics of included studies in Ardabil province, Northwestern Iran, 1994-2018

Reference	*Year*	*County*	*Dogs*			*Male*		*Female*	
			Total	Positive	%(DAT)	No.	%	No.	%
Bokaii et al (19)	1994	Meshkinshahr	303	45	14.8	235	77.5	68	22.5
Mohebali et al (32)	2001	Meshkinshahr	344	17	4.9	237	69	107	31
Gavgani et al (21)	2002	Meshkinshahr	199	43	21.6	115	58	84	42
Sadeghi et al (33)	2003	Parsabad Meshkinshahr	458	34	7.4	350	76.5	108	23.5
Mohebali et al (34)	2004	Meshkinshahr	268	106	39.5	205	76.5	63	23.5
Mohebali et al (35)	2004	Meshkinshahr	303	23	7.59	228	75.2	75	24.8
Mahami et al (36)	2005	Germi	22	3	13.7	14	63.6	8	36.4
Mohebali et al (23)	2005	Meshkinshahr	916	167	18.2	632	69	284	31
Mohebali et al (37)	2006	Meshkinshahr, Ardabil Parsabad, Khalkhal	290	30	10.3	226	78	64	22
Taran et al (38)	2007	Meshkinshahr	126	55	43.6	95	75	31	25
Moshfe et al (24)	2008	Meshkinshahr	384	67	17.4	290	75.5	94	24.5
Moshfe et al (39)	2009	Meshkinshahr	66	16	24.2	41	62	25	38
Salahi-Moghaddam et al (40)	2010	Meshkinshahr	384	35	10	270	70.3	114	29.6
Sharifdini et al (41)	2011	Meshkinshahr	171	27	15.8	146	58.4	25	14.6
Mohammadi et al (26)	2011	Meshkinshahr	60	7	11.6	40	66.7	20	33.3
Mohebali (31)	2013	Meshkinshahr	3308	608	18.4	2315	70	993	0
Mohammadiha et al (42)	2013	Meshkinshahr	100	28	28	80	80	20	20
Shabestariasl et al (43)	2013	Meshkinshahr	100	45	45	75	75	25	25
Jalilnavaz et al (44)	2014	Meshkinshahr	118	20	15.8	90	76.2	28	23.8
Barati et al (27)	2015	Meshkinshahr	508	119	23.4	397	78	111	22
Ghaffarinejad et al (45)	2015	Meshkinshahr	200	49	24.5	164	82	36	18
Farahmand et al 46)	2015	Meshkinshahr	350	91	26.9	265	76	85	24
Molai et al (30)	2016	Meshkinshahr	110	36	32.7	89	80.1	21	19.9
Total			9088	1671	14.56	6599	72.6	2489	27.4

The first study on dogs in Meshkinshahr was carried out in 1994 and the prevalence of CVL was found to be 14.8% (19). Moreover, according to the latest study in 2016, CVL infection was 32.7% in dogs (30). Over the past 23 years, the prevalence of CVL in dogs has increased up to 17.9%. In Ardabil Province, the highest (3308 cases) and lowest (60 cases) number of CVL-infected dogs were studied in 2013 and 2011, respectively (26, 31). These two studies were conducted in 6 counties and 119 areas of the province. Generally, 81.5% of studies were done in rural and urban areas, and 18.5% were conducted in tribal regions. The studies included sex, age, location, and clinical symptoms (Tables 2,3). The prevalence of CVL in male dogs was higher than females in all previous studies (Table 2). As far the age, an increase in the age of the dogs significantly raised the prevalence of the disease, and in dogs which were 5 year or older, the prevalence was about 4 times higher than other age groups (Table 3). More than 56% of the studied dogs lived in rural areas, and the infection rate in rural and urban areas were 19.14% and 14.33%, respectively (Table 4). The results of the MaxEnt model indicated that the presence probability of a large section of the province was less than 20%, and the most ecologically suitable areas of CVL occurrence were identified in four hotspots in Meshkinshahr, Germi, and two spots of Parsabad with a population of 732,110 at risk (Fig. 2). The areas under the receiver operating characteristic (ROC) curve (AUC) were 0.945 and 0.885 for training and test data, respectively. According to the Jackknife test, the Isothermality was found to be the highest gained environmental variable when used in isolation (Table 5). In a way, that higher Isothermality values had a positive effect on the presence probability of CVL. The Bio3, Bio13 and Bio4 variables were the other environmental factors with highest contribution to the model (Fig. 3).

Table 2: Prevalence of *Leishmania infantum* infection in dogs by sex in Ardabil Province, Northwestern Iran

References	*Province*	*City*	*Total*	*Total Infected*	*Male*			*Female*			*P-value*
					No. examined	**No. infected**	**Infected %**	**No. examined**	**No. infected**	**Infected %**	
Mohebali et al. (2001) (32)	Ardabil	Meshkinshahr	344	17	237	14	5.9	107	3	2.8	0.024
Mohebali et al. (2004) (34)	Ardabil	Ardabil	268	106	210	ND	ND	67	ND	ND	ND
Mohebali et al. (2005) (23)	Ardabil	Meshkinshahr	916	129	632	95	15	284	34	12	0.003
Moshfe et al. (2008) (24)	Ardabil	Meshkinshahr	384	67	290	48	16.5	94	19	20.2	0.049
Sharifdini et al. (2011) (41)	Ardabil	Meshkinshahr	171	27	146	24	16.4	25	3	12	0.000
Barati et al. (2015) (27)	Ardabil	Meshkinshahr	508	119	397	101	25.4	111	18	16.2	0.001
Total			2591	465	1912	268	14	688	74	10.75	0.089

ND: No data.

Table 3: Prevalence of *Leishmania infantum* infection in dogs by age groups in Ardabil province, Northwestern Iran

References	*Total Dogs*	*Age Group (yr)*	*Dogs*			*P-value*
			Number of examined	**Number of infected**	**Infected %**	
Mohebali et al. (2005) (23)	916	0-3 yr	542	54	10	0.047
		4–7 yr	300	45	15	
		≥ 8 yr	74	30	40.5	
Moshfe et al. (2008) (24)	384	0-3 yr	239	24	10	0.049
		4–7 yr	112	30	26.8	
		≥ 8 yr	33	13	39.4	
Sharifdini et al. (2011) (41)	171	< 2year	52	3	5.8	0.005
		2–5 yr	83	15	18.1	
		>5 year	36	9	25	
Barati et al. (2015) (27)	508	<2 yr	164	29	17.7	0.001
		2–5 yr	264	67	25.4	
		≥ 5yr	80	23	28.7	
Total	1979	-	1979	342	17.3	-

Table 4: Prevalence of *Leishmania infantum* infection in dogs according to settled in Ardabil province, Northwestern Iran

References	*Total examined Dogs*	*Infected dogs*	*Urban dogs*		*Rural dogs*		*P-value*
			No. of examined	**Infected %**	**No. of examined**	**Infected %**	
Mohebali et al. (2001) (32)	29	22	20	69	9	31	0.002
Mohebali et al. (2005) (23)	1568	222	678	30.6	890	69.4	0.037
Moshfe et al. (2008) (24)	384	67	384	100	0	0	-
Moshfe et al. (2009) (39)	66	16	66	100	0	0	-
Mohammadi-Ghalehbin et al. (2011) (26)	60	2	60	100	0	0	-
Sharifdini et al. (2011) (41)	171	27	0	0	171	100	-
Barati et al. (2015) (27)	508	119	0	0	508	119	-
Total	2786	475	1208	36.4	1578	63.6	-

Table 5: Variables contribution is MaxEnt modeling for predicting distribution of VL infection in dogs, Ardabil Province, Northwest Iran

Variable	*Description*	*Contribution (%)*
Bio1	Annual mean temperature (°C)	0
Bio2	Mean diurnal range: mean of monthly (max temp–min temp) (°C)	1.4
Bio3	Isothermality: (Bio2/Bio7) × 100	44.4
Bio4	Temperature seasonality (SD × 100)	14.6
Bio5	Maximum temperature of warmest month (°C)	0
Bio6	Minimum temperature of coldest month (°C)	6
Bio7	Temperature annual range (Bio5 − Bio6) (°C)	2.2
Bio8	Mean temperature of wettest quarter (°C)	0.7
Bio9	Mean temperature of driest quarter (°C)	0
Bio10	Mean temperature of warmest quarter (°C)	0
Bio11	Mean temperature of coldest quarter (°C)	0.6
Bio12	Annual precipitation (mm)	2.9
Bio13	Precipitation of wettest month (mm)	15.2
Bio14	Precipitation of driest month (mm)	4.6
Bio15	Precipitation seasonality (coefficient of variation)	1.1
Bio16	Precipitation of wettest quarter (mm)	0.9
Bio17	Precipitation of driest quarter (mm)	1.7
Bio18	Precipitation of warmest quarter (mm)	0.1
Bio19	Precipitation of coldest quarter (mm)	2.2
Altitude	Elevation from the sea level (m)	1.1
Slope	Slope of the area (%)	1.7
Aspect	Direction of slope (Degree)	2.9
NDVI	-1 to +1	5.2

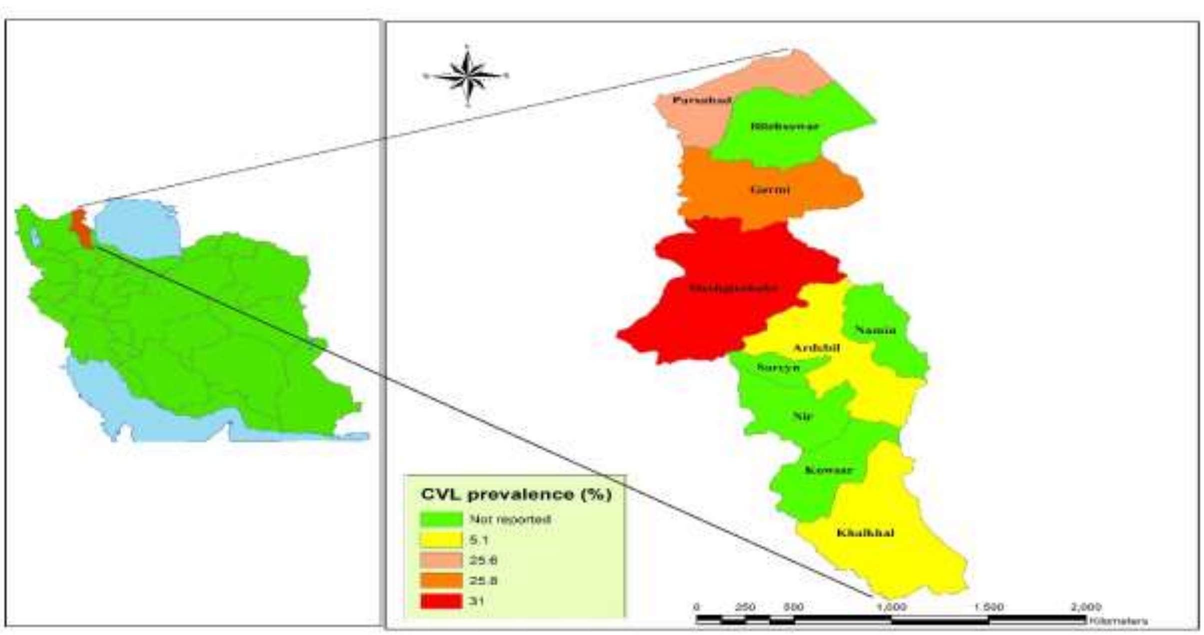

Fig. 1: Study area and prevalence of canine visceral leishmaniasis in Ardabil Province, Northwest Iran during1994-2018

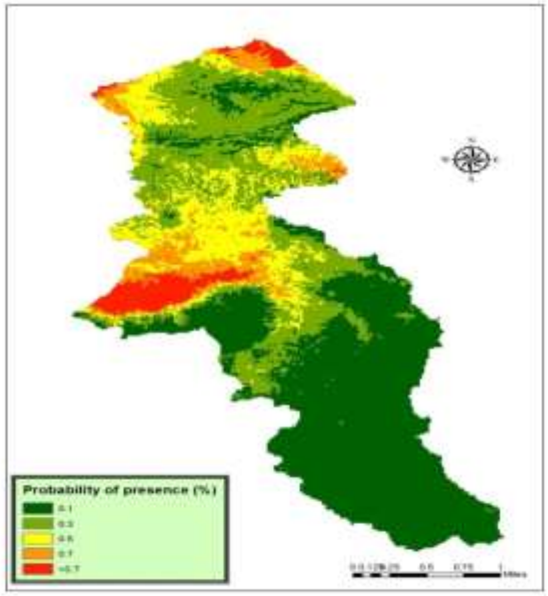

Fig. 2: Ecologically suitable areas for occurrence of infected Dogs to *Leishmania infantum* in Ardabil Province, Northwest Iran

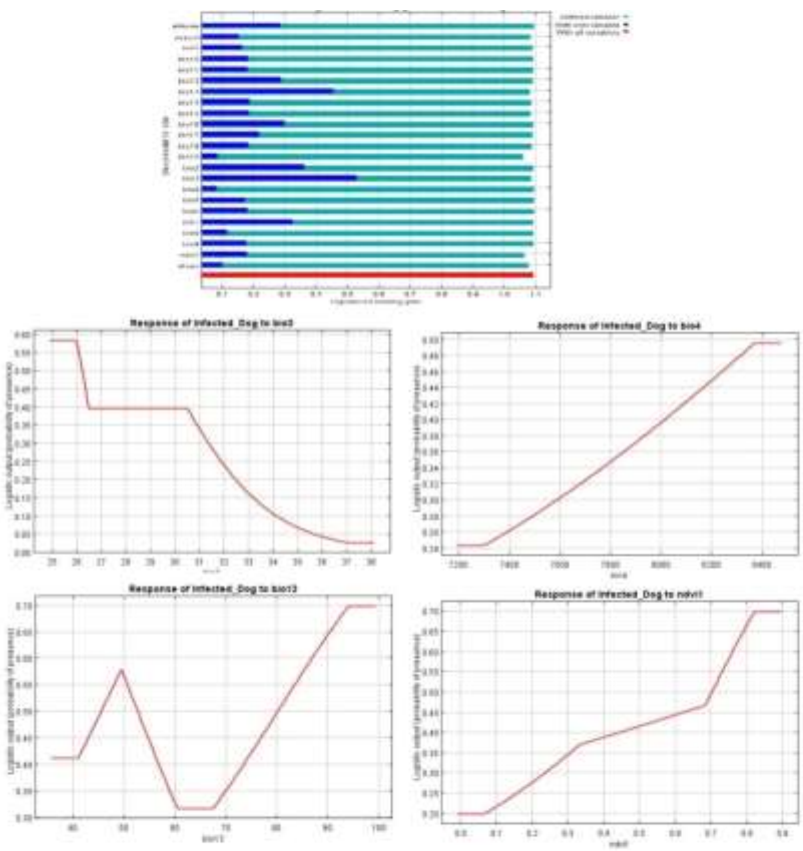

Fig. 3: Result of jackknife test of variables importance for infected Dogs to *Leishmania infantum* in Ardabil Province, Northwest Iran

Discussion

The VL is a zoonotic disease in tropical and subtropical regions seen in humans and dogs (13). Dogs are the main reservoir for human infections in most areas of the world; (11) in endemic areas of Iran, domestic dogs are one of the risk factors of developing HVL (21). Ardabil Province is one of the most important high-risk foci in terms of HVL and CVL (2). Twenty-three studies were investigated using DAT test (Cut off, ≥1/320) in Ardabil Province in 1994-2018. The results of this review showed that 9088 stray and domestic dogs had been tested. Of these studies, 1671 dogs were founded to be infected using DAT test. In Iran, out of 19903 dogs were examined in many areas in 1982-2014, 2464 dogs were tested positive by the DAT test by different cut-off points (47). The frequency of infected dogs in Ardabil Province was 14.56% (95% CI: 4.7-45 %) in average, and 16.4% (95% CI:15-20%) in the whole country in the previous 23 years (4). Several studies have been conducted in other regions in Iran and have reported that the numbers of infected dogs in Fars (48), and Alborz Provinces (49, 50) have been 26.3%-30%, 34.6%, and 3.6%-4.98%, respectively. The CVL studies in other regions of the world were done using different laboratory methods. For instance, the result of a study on infection rate of dogs in Brazil by PCR method in 2006 showed the infection rate was 5.8% (95% CI: 5.1-6.5%) (51), 8.4% in east Amazon (52), and 40.2% in Brazil (53). The prevalence of CVL in Meshkinshahr County was more than in other regions in Ardabil Province. Because most HVL infections in recent years have been reported from Meshkinshahr County in Ardabil Province(31, 54, 55), most of the studies on reservoir hosts have focused this region and have observed that more dogs are being infected with *L. infantum*. Our results reveal that male dogs have been studied more than female ones in in Ardabil Province showing that the maintenance of the male dogs' population is more than female counterparts. The results of this review is in agreement with the findings from other regions in Iran. For example, in Khuzestan, 54% of dogs were males and 46% of them were females (56). Likewise, in East Azerbaijan, 80% of dogs were males and the rest were females (57). In addition, in Kerman, south of Iran, 67% of cases were males (58). The result of another study from 2008 to 2014 in Brazil showed that 71% of the dogs were males (59). The results of DAT test showed that the prevalence of *L. infantum* in male dogs was more than females. Other findings (60-62) were also in line with the findings of this study. The results of the present study showed that 5-year-old and older were four times more infected than dogs below that age. This finding is similar to the reports from East Azerbaijan Province (57). Thus, older dogs are more susceptible to exposure to sandfly bites in the long run. Some studies in Ethiopia (63) and Portugal (64) also observed similar results, but in Khorassan Razavi (65) and Hamadan provinces in northeast and west of Iran (66) opposite results were observed as dogs younger than 2 year were more infected. This difference may be because of different geographic regions in northwestern Iran and other areas. According to the results of this study, CVL infection in dogs, as a result of *L. infantum*, in rural areas was more than urban areas. Visceral leishmaniasis is directly related to the humans occupation (6). Since most of the people who reside in rural areas and villages of Ardabil province are ranchers and farmers, they have to keep stray dogs in order to protect their domestic animals.

Accordingly, in other parts of Iran, for example, in Hamadan (66), and Kerman (58), the CVL infection rate in dogs in rural areas were more than urban areas. These results indicate a direct relationship between occupation and CVL. This study showed that the prevalence of CVL in Ardabil Province over the past years has been slowly increasing. These results were obtained through the DAT method, and the actual prevalence in all dogs living in this area is not clear; similarly, other areas of the province (e.g., southern regions) have not been studied so far. Hence, a comprehensive study on symptomatic and asymptomatic dogs should be conducted, and the probable factors associated with the increasing prevalence of *L. infantum* in dogs should be investigated.

Moreover, the results of MaxEnt analysis showed that the most important environmental and climate factors affecting CVL infection rate in dogs include Isothermality, temperature seasonality, precipitation of wettest month, and NDVI. In Brazil, the most frequent cases of CVL occurred in those places which had high-density vegetation, rivers, and canals, as well as those homes with lots of vegetation and debris (49). Nevertheless, in France, there was a negative correlation between the NDVI factor and CVL cases and the result of relevant MaxEnt model in CVL showed that five variables had significant impacts: average summer rainfall, average annual temperature, average winter minimum temperature, and the percentage of surface covered by coniferous forest, and altitude (67).

Conclusion

In Iran, CVL in dogs has not been modeled so far, and the current study was the first attempt to investigate the occurrence of CVL by MaxEnt model. Nevertheless, the other main reservoirs of cutaneous leishmaniasis have been studied in Iran. A widespread epidemic is emerging among dogs, posing grave risks to the inhabitants of these areas and increasing the probability of an outbreak of HVL. Therefore, the findings of this study present a bird's-eye view of general situation of CVL over the past 23 years so that an appropriate decision is taken to prevent and control VL in Ardabil Province.

Ethical considerations

Ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

Authors declare that there is no competing interest.

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